SUPPLEMENTARY MATERIALS

Figure S1, Related to Figure 1. NP deficiency does not affect islet number, size and beta cell mass. (**A**) Bar graph analysis of the number of islet equivalents (IEQ) in isolated islets from NP deficient mice. (**B**) Bar graph analysis with the distribution of islet size in isolated islets from NP deficient mice. Categories of islet size were divided as 50-100 mM (black), 100-200 mM (dark grey) and 200-250 mM (clear grey). (**C**) Bar graph analysis of beta cell mass (mg) in isolated islets from NP deficient mice. (**D**) Representative western blot analysis and bar graph analysis of NP expression in brain protein lysates. Data represent the means ± S.E.M., *n=5* mice per genotype, except for **D** where *n=3* was utilized.

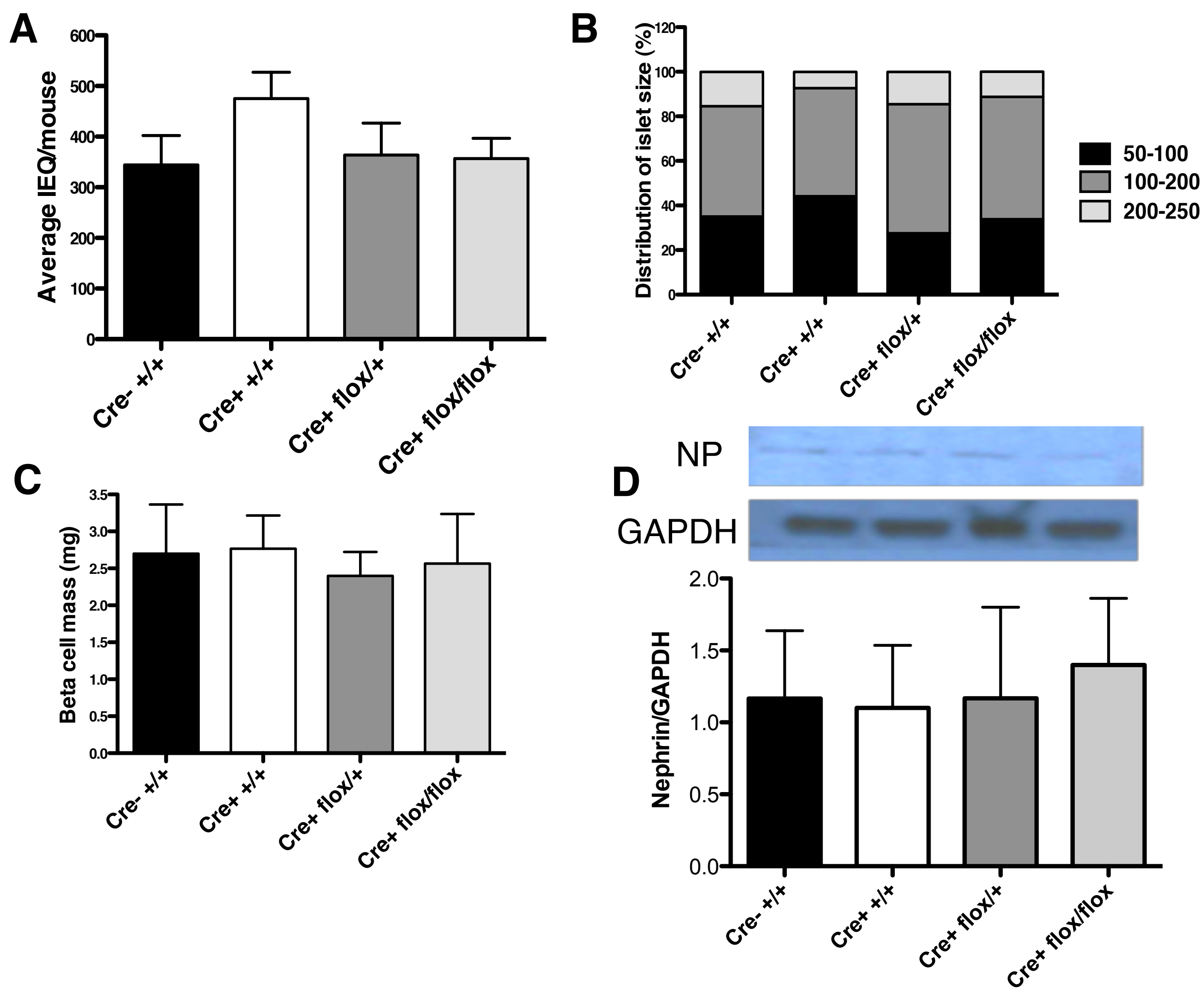
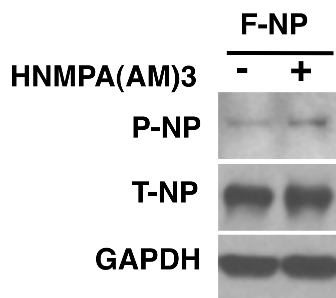


Figure S2, related to Figure 4. Inhibition of IR phosphorylation did not affect NP phosphorylation. (**A**) Representative WB showing Y1176/1193 NP phosphorylation levels in F-NP transfected HEK293 cells at baseline and after pre-incubation for 60 min with HNMPA(AM)3 compared to control. (**B**) Bar graph analysis of phosphorylated NP (P-NP) when compared to control. Data represent the means ± S.E.M., $n=6$. Each value was corrected by the value of total protein (T-NP) and GAPDH.



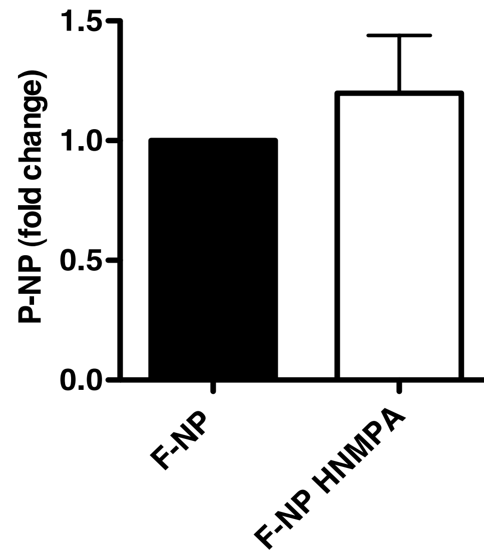


Figure S3, related to Figure 4. Src kinase inhibition does not affect NP and IRB interactions. (**A**) Representative WB for FLAG and GFP from lysates (Input) and immunoprecipitates (IP) from HEK293 cells transfected with F-NP and G-IRB after 60 min of pre-incubation with 10 mM of Src inhibitor PP2 and stimulated or not with 1 nM insulin for 20 min. (**B**) Representative WB showing Y1176/1193 NP phosphorylation (P-NP) levels in F-NP transfected HEK293 cells at baseline and pre-incubated 60 min with 10 mM of Src inhibitor PP2 compared to control (upper panel). Bar graph analysis when comparing PP2 pre-incubated cells to control (lower panel). Data represent the means ± S.E.M., *n=3 ** P<0.01* when compared to control. Each value was corrected by the value of total protein (T-NP) and GAPDH.

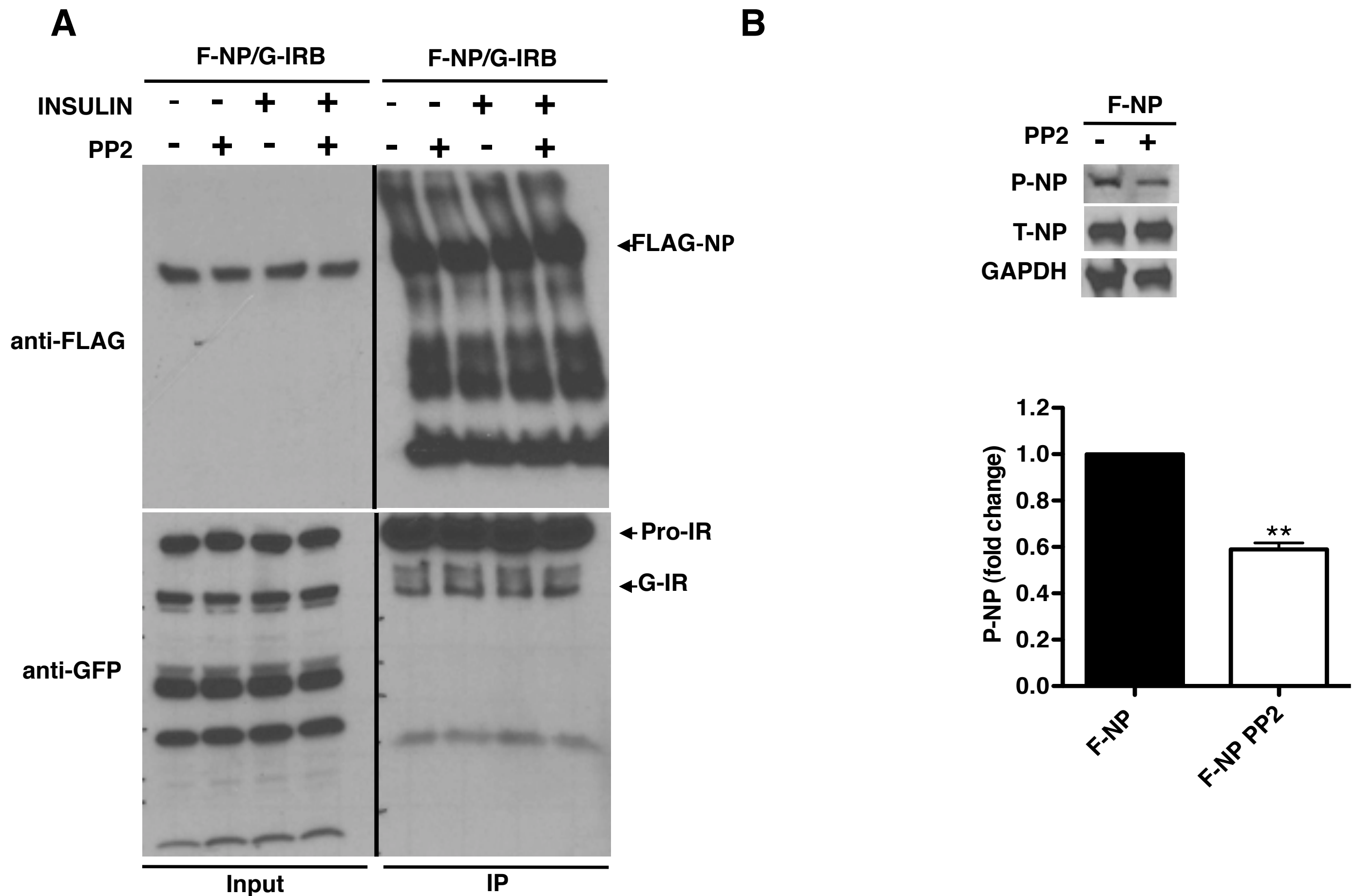


Figure S4, related to Figure 7. 3YF NP mutant can phosphorylate p70S6K. 3YF NP mutant can phosphorylate p70S6K. WB showing p70S6K phosphorylation levels in HEK293 lysates from cells transfected with either F-NP WT or Flag-tagged Y1176F/1193F/Y1217F NP mutant at baseline. Bar graph representation of the three experiments.

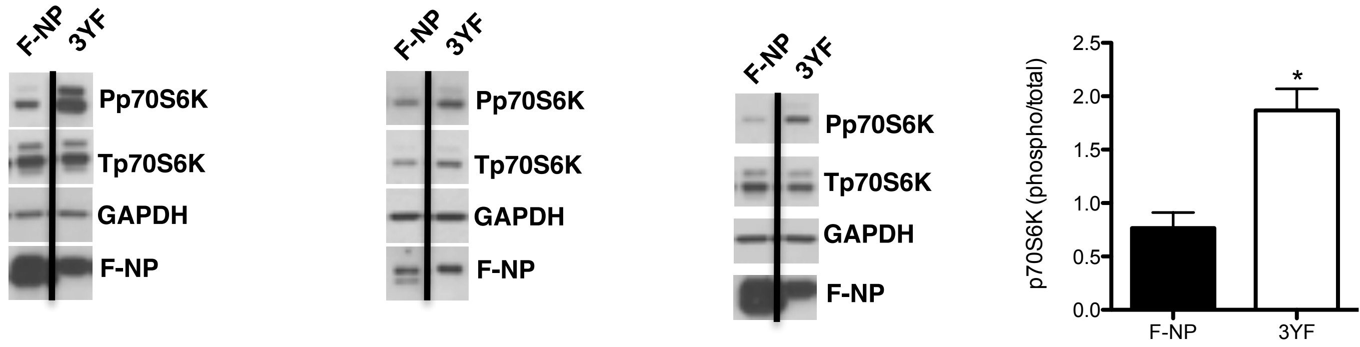
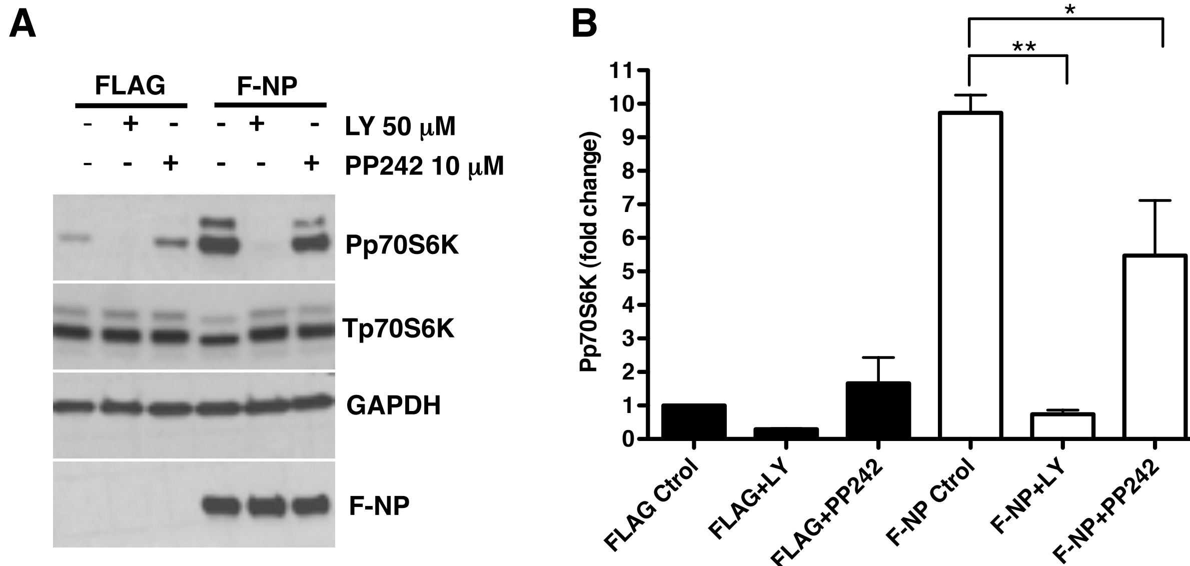


Figure S5, related to Figure 7. PI3K and mTORC2 inhibition decrease NP phosphorylation. (**A**) Representative WB of FLAG or F-NP transfected HEK293 cells pre-incubated 60 min with insulin signaling inhibitors as indicated: PI3K inhibitor LY294002 (LY) and mTOR inhibitor PP242 at baseline and at indicated concentrations. (**B**) Bar graph analysis of Pp70S6K in FLAG and F-NP transfected cells exposed to the different inhibitors. Each value was corrected by the value of total protein (Pp70S6K) and GAPDH. Data represent the means ± S.E.M., *n=3 * P<0.05* when compared PP442 treated versus untreated F-NP transfected cells*,* ** *P<0.01* when compared LY treated versus untreated F-NP transfected cells.



Methods and Figure S6

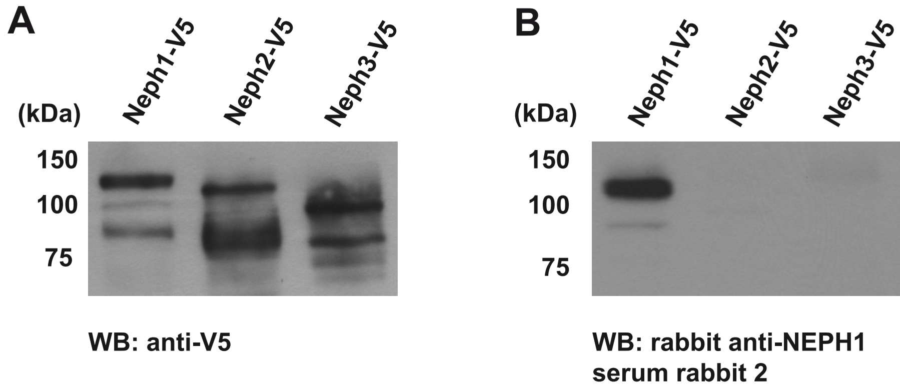
NEPH1 Antibody Generation

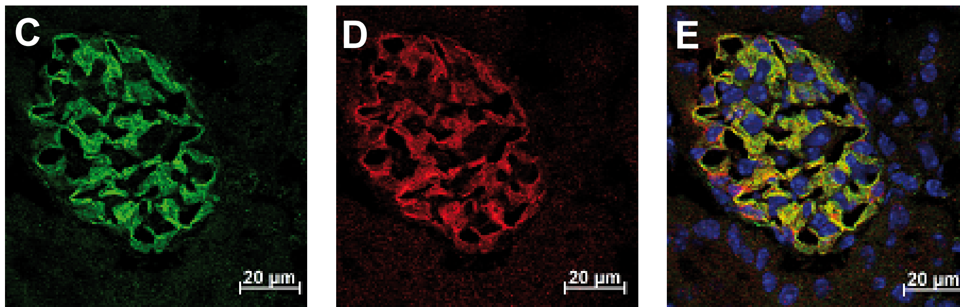
We generated a NEPH1 peptide-antibody against mNEPH1 aa 767-788 by immunizing a rabbit with the corresponding peptide coupled to Keyhole Limpet Hemocyanin using Freud's complete adjuvant on d1 and Freund's incomplete adjuvant on d20, d30 and d40. From d61 a boost was given every 15 days. The final bleed was performed on d130 after a positive immunoreactive test bleed on d120, and the serum affinity was purified against the immunogenic peptide used (Pineda Antikörper, Berlin, Germany).

HeLa cells (ATCC, Manassas, Virginia, USA) were transfected with *mNeph1*, *hNeph2* and *hNeph3* using Lipofectamin (Life Technologies, Karlsruhe, Germany)(1). Cell lysis was done in lysis buffer (containing 20 mM CHAPS and 1% Triton X-100). After centrifugation (15,000xg, 15 min, 4°C), protein concentration was determined by DC Protein-Assay (Bio-Rad, Munich, Germany). Equal amounts of protein were separated on SDS page. HRP coupled 2nd antibodies and ECL in combination with a conventional x-ray system (films: Fuji, Tokio, Japan; developer: AGFA, Mortsel, Belgium) were used to detect western blot bands. Mouse anti-V5 1:5000 (Life Technologies, Karlsruhe, Germany) was used to proof expression of transfected proteins (Figure S6A). Rabbit anti-NEPH1 antibody was used 1:1000 to demonstrate specificity for Neph1.

For immunofluorescence kidneys were frozen in OCT compound and sectioned at 5 µm (Leica Kryostat, Wetzlar, Germany). The sections were fixed with 4% paraformaldehyde in phosphate

buffered saline (PBS), blocked in PBS containing 5% BSA + 5% Normal Donkey Serum (Jackson Immuno Research, Suffolk, UK) and incubated for 45 min with rabbit anti-NEPH1 (Figure S6C) and guinea pig anti-NEPHRIN (Figure S6D, Progen, Heidelberg, Germany) as indicated. After several PBS rinses, fluorophore-conjugated secondary antibodies (Life Technologies, Darmstadt, Germany) were applied for 30 min. Images were taken using a Zeiss fluorescence microscope equipped with a 63x water immersion objective (Zeiss, Oberkochen, Germany). Colocalization is shown in Figure S6C.





References

1. Sellin, L., Huber, T.B., Gerke, P., Quack, I., Pavenstadt, H., and Walz, G. 2003. NEPH1 defines a novel family of podocin interacting proteins. *FASEB J* 17:115-117.